CL. PROTEIN AND VITAMIN B.

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BOTH protein and vitamin B¹ are accepted as essential constituents of the diet, but the amount of protein which can be metabolised without harmful results is a question on which there is a variety of opinions. For some time it was thought that a high protein diet produced nephritis, but more recent work seems to show that an animal can tolerate large proportions of protein in its diet. Osborne and Mendel [1924] succeeded in feeding rats on a diet containing as much as 80 % caseinogen, while Osborne, Mendel, Park and Winternitz [1926] gave a diet consisting of 95 % caseinogen and found that the rats appeared fit and well. These observers obtained good growth to adult size with rats on high protein diets, but noticed hypertrophy of the kidneys, when the protein comprised one-third or more of the total ration.

Addis, MacKay and MacKay [1926] find that rats can "live for a third of their life-span on a diet very high in casein (70 %) or with added cystine, and yet escape any damage to their kidneys." Jackson and Riggs [1926] fed rats on diets containing 76 % caseinogen, and 20 % caseinogen + 56 % egg-albumin; they found hypertrophy of the kidneys, but no nephritis. The success of these various workers in feeding high protein diets suggests that the failure of others with similar diets is due, not to the high proportion of protein itself, but rather to a deficiency of some other dietary factor or to a lack of balance between some of the constituents. There is evidence that vitamin B is closely related with protein metabolism.

Hartwell [1922, 1, 2, 1924, 1925, 2] has shown that there is a definite relation between the amount of vitamin B and protein in the diet of the lactating rat. Reader and Drummond [1925] found that rats fed on a diet containing 20 % caseinogen grew to adult size, and attained a weight of over 300 g., but when the protein was raised to 45 % none of the rats reached 250 g., while on 90 % of caseinogen no rat grew to 150 g. The animals appeared to be in good health, and *post mortem* examinations showed nothing abnormal, except hypertrophy of the kidneys. In a later paper [1926] these observers confirmed this result, and further found that in order to obtain good growth on such a diet the proportion of yeast extract (vitamin B) must be raised so

¹ By the term vitamin B is meant the original water-soluble B which has now been shown to consist of at least two factors.

that "the ratio of protein to yeast has a value 5 or under." Thus there is evidence that for maximum growth the vitamin B of the diet must be increased when the protein is increased. The proteins used in experiments quoted above were caseinogen, and a mixture of caseinogen and egg-albumin. Bond [1922] obtained good growth in rats on a diet containing 30 % of egg-albumin and 5 % marmite. This relation between protein *qua* protein and vitamin B suggests that there may be some proteins which will require more vitamin B than others for their metabolism, since proteins vary considerably in their composition. Experiments to be described in this paper demonstrate that for normal metabolism edestin requires far more vitamin B than does caseinogen or egg-albumin.

EXPERIMENTAL.

Piebald rats bred in the laboratory were used. Twelve rats, 6 ♀ and 6 ♂, were used in each experiment, except where otherwise stated, and each experiment was started when the rats weighed approximately 40 g., except Exp. 11. The basal diet consisted of

20 g. edestin¹.
64 g. potato starch.
12 g. butter.
4 g. salt mixture [Hartwell, 1922, 1].
300 cc. distilled water.

The food was made freshly every day except Sunday, the "cooked" method being used [Hartwell, 1925, 1]. The same sample of marmite was used throughout. The rats were allowed food *ad lib.*, and distilled water to drink. They were weighed daily for the first 4 weeks, and afterwards the weighing was omitted on Saturdays and Sundays. The animals were not kept on screens (except for part of Exp. 9) because this work was started in 1925 when the necessity for such a precaution was not realised. The floor of the cage was covered with sawdust to which the animals had access. All the experiments to be described in this paper were made under the same conditions and are therefore comparable. The growth curves are given in Fig. 1.

Exp. 1. Basal diet + 2 g. marmite.

After about a week several of the rats became weak and were obviously ill, 5 of them died on the 13th day, having shown a previous loss in weight. The remaining 7 were killed and *post mortem* examination showed only one to be normal, while the other 6 had kidneys of a deep purple colour and gorged with blood. (The histological details will be described in a later paper.)

Exp. 2. Basal diet + 2 g. marmite.

The results described above were so striking that it was thought advisable to repeat the experiment. Accordingly 12 more young rats were fed on a similar diet, and 6 died by the 14th day; 4 more were killed and each showed the typical purple kidneys. The remaining 2 were kept for 9 weeks on the diet

¹ The edestin used in these experiments was made by Messrs E. T. Pearson and Co. Ltd.

and were then killed. Their kidneys were slightly yellowish, but otherwise normal.

Fig. 1. Growth curves of rats on synthetic diets.

* Diets described by Hartwell [1927].

† " " [1926].

K Rat killed.

Exp. 3. Basal diet + 2 g. marmite.

It was thought that the above results might be due to some alkaloid adhering to the edestin, and therefore a new sample was prepared by the

method of Osborne and Mendel [1894]. The results appeared little better, only 2 rats out of 12 showing normal kidneys after 5 weeks on the diet. Three animals died in 14 days and one was killed after a bad loss in weight; this rat had the typical purple kidneys.

Exp. 4. Basal diet + 2 g. marmite.

In the next experiment the edestin used was subjected to several hours' further washing in cold water, in the hopes of removing the hypothetical impurity. Two rats died in 14 days and 4 others which showed the typical loss in weight were killed; all 4 had purple kidneys. After 4 weeks on the diet, the remaining 6 were killed and 5 appeared to be normal. These results were certainly better, but as half the rats developed abnormal kidneys, the edestin could still not be regarded as a satisfactory food protein. It was therefore decided to try edestin which had been extracted with alcohol and if that failed to try extraction with ether.

Exp. 5. Basal diet + 2 g. marmite.

The edestin was extracted with 93.58 % ethyl alcohol by weight, the extraction being repeatedly carried on for a period of 3 weeks.

Of the 12 rats used in this experiment, 1 died within a week and 2 more on the 18th day, at which time 4 others which showed loss of weight were killed; all 7 showed the typical purple kidneys. The remaining 5 animals were killed after 5 weeks on the diet, and only one had normal kidneys.

Exp. 6. Basal diet + 2 g. marmite.

The edestin used in this experiment was extracted with alcohol as in *Exp. 5* and then further extracted with ether repeatedly for a period of 2 weeks.

After 2 weeks on the diet 1 rat was eaten, and therefore 5 others which had lost weight were killed; each had enlarged and purple kidneys. The other 7 rats were kept for $3\frac{1}{2}$ weeks on the diet and then killed; macroscopically 5 appeared to have normal kidneys.

Exp. 7. Basal diet + 2 g. marmite + 3 cc. extract containing vitamin B [Hartwell, 1922, 2].

In view of these results, it seemed unlikely that any poisonous substance adhering to the protein was responsible for the bad effects and therefore it was thought advisable to alter the proportions of other constituents of the diet. To the basal diet + 2 g. marmite were added 3 cc. of an extract containing vitamin B ($\equiv$ 3 g. marmite). On this diet 7 rats died in 14 days, at the end of which time the other 5 were killed, only 2 of them showing normal kidneys.

Exp. 8. Basal diet + 15 g. marmite.

18 rats were used in this experiment and 15 g. marmite were given. With this addition to the diet, the growth curves were good and no loss in weight occurred. Six rats, 3 ♀ and 3 ♂ were killed after 2, $3\frac{1}{2}$ and 5 weeks respectively on the diet, and each animal had normal kidneys, thus it was obvious that the yeast extract had prevented the bad symptoms.

Exp. 9. Basal diet + 15 g. marmite (modified after 4 weeks).

Exp. 8 lasted only 5 weeks and it was decided to repeat it and continue

the diet for a longer period in order to see if the improvement were temporary or permanent, since the addition of marmite might have delayed, but not prevented, the kidney symptoms. The basal diet + 15 g. marmite was given for 4 weeks and after this the rats were placed on screens in order that the work might be comparable with another series of experiments. At the same time the basal diet was modified so that the mineral salts were increased, 8 g. instead of 4 g. being used, while the starch was correspondingly lowered to 60 g. instead of 64 g. This modification was introduced because there was some indication in another experiment that the proportion 4 % of mineral salts was rather low for breeding. On this modified diet the rats lived for 12 weeks. All the animals were in excellent condition, their fur was thick and silky and did not come out at any period. The modification in diet and technique introduced at the end of the 4th week made no appreciable difference to the growth curve. During the experiment 5 does each produced 2 litters and the 6th one had 3 fetuses in the uterus when killed. *Post mortem* examination showed that all organs and tissues were macroscopically normal, except for the thyroids which were slightly enlarged.

Exp. 10. Basal diet + 10 g. marmite.

An experiment was next made using only 10 g. marmite to see if this quantity were adequate. After 4 weeks on the diet all 12 rats appeared fit and well and their growth curves were good, in fact the animals grew at the same rate as when they received 15 g. marmite. Accordingly 2 ♀ and 2 ♂ were killed and as *post mortem* examination revealed no abnormalities, the other rats were not killed.

Exp. 11. Basal diet + 2 g. marmite (older rats).

The next problem was to see if rats of all ages developed kidney symptoms on the edestin diet and therefore an experiment was made using partly grown rats. Six males ranging from 98 to 145 g. and 5 females from 85 to 110 g. were used. From weaning until given the edestin diet, the rats were treated as stock animals, *i.e.* they received a little bread and milk every day and kitchen scraps *ad lib.* After 14 days on the edestin diet, 2 ♀ and 2 ♂ were killed; their kidneys appeared quite normal. After 6 weeks, 2 ♀ and 2 ♂ were killed; and as they also were normal in all respects, the experiment was discontinued. One doe produced a litter of 6 and reared 4 of them (but the marmite was increased during the lactation period). All the rats grew steadily and well, though less rapidly than on a control diet of kitchen scraps.

Exp. 12. Basal diet + 15 g. marmite autoclaved at 120° for 4½–5 hours.

(a) This diet was given for 17 days during which time the rats grew well and appeared to be in good condition. Since the loss in weight and death of some animals was usually noticed before the 14th day, it seemed that these rats were safely over the danger period, but 4 were killed to see if the kidneys were abnormal; all 4 however appeared quite normal.

(b) A second experiment was made in which the rats were kept on the basal diet + autoclaved marmite for 6 weeks. During this period the growth

curves were good and steady, no loss in weight being shown. The rats were all in excellent condition at the end of the 6 weeks, therefore the factor in marmite which prevented the kidney symptoms had not been destroyed by the high temperature.

Previous experiments with other proteins have shown good growth with 5 g. or less of marmite in 100 g. dry solid food. Hartwell [1926] obtained steady growth in rats on a diet containing 20 % caseinogen and only 2 g. marmite, and quite good growth with the equivalent of 5 g. marmite and a mixture of "food casein" and gluten. Later [Hartwell, 1927] caseinogen + egg-albumin, and caseinogen + lactalbumin produced satisfactory growth when 5 g. marmite were added to 100 g. solid. Thus it appeared that the metabolism of edestin required more marmite than other proteins and in order to test this hypothesis further, experiments were made with synthetic diets containing 20 % of egg-albumin with 5 g. and 2 g. marmite respectively.

Exp. 13. Basal diet in which edestin was replaced by egg-albumin + 5 g. marmite.

Twelve rats were fed on this diet for 4 weeks; growth was good and the animals were fit and well throughout the experiment. *Post mortem* examination of 3 ♀ and 3 ♂ showed normal kidneys.

Exp. 14. Basal diet as in *Exp. 13* + 2 g. marmite.

In this experiment 6 ♀ and 5 ♂ rats were used; they grew well during 4 weeks on the diet. Then 2 ♀ and 2 ♂ were killed, but nothing abnormal was seen.

RESULTS.

1. *Edestin experiments.*

A synthetic diet containing 20 % of edestin as the sole protein and 5 g. or less of marmite per 100 g. solid is not a satisfactory food for young rats. Many of the animals die during the first 2 weeks of the experiment, with a characteristic drop in weight before death. Other rats show a similar depression of the weight curve (Fig. 1), but eventually recover. The animals which die become limp and cold, with sunken eyes, but otherwise no external symptoms can be detected. There is no paralysis as with a vitamin B-free diet. *Post mortem* examinations were made on 84 rats and of these only 18 showed normal kidneys. The typical depression in the weight curves is indicative of abnormal kidneys. Partly grown rats can live on a diet of 20 % edestin and 2 g. marmite, and do not develop kidney symptoms.

2. *Amount of yeast extract in relation to kind of protein.*

With egg-albumin, the addition of 5 g. marmite instead of 2 g. per 100 g. dry solid made no appreciable difference in the growth curves of the rats, but a corresponding alteration in the caseinogen diet led to a marked improvement in the rate of growth. With edestin on the other hand, 10 g. of marmite were needed to provide for good growth.

3. *Rate of growth.*

When egg-albumin was used as the sole protein, the rate of growth was just slower than with either of the other proteins, but the curves were quite good, and the rate of growth steady. With edestin, the growth was equal to that obtained with caseinogen, provided a larger proportion of marmite were added to the basal diet. There appeared to be no difference in the rate of growth of the rats on the edestin diet when they were given 15 g. or 10 g. yeast extract, but with the autoclaved marmite (15 g.) the rate of growth was a little slower.

COMMENTS.

It seems unlikely that the edestin in itself is responsible for the bad effects obtained in these experiments, because this protein has been used successfully by other nutrition workers for a considerable time. Osborne and Mendel [1916] fed rats on a diet containing 15 % of edestin for 11 weeks, and in other experiments [1919] they used 17.9 % of edestin for a period of 8 weeks. Osborne, Mendel, Park and Winternitz [1926] obtained good growth to adult size with 16 % of edestin, while Osborne and Mendel [1924] were successful in feeding rats on a diet containing as much as 50–55 % edestin. This latter work is very interesting in that Osborne and Mendel state "several attempts to raise animals on fat-free diets containing 95 % of protein of which 90 % was edestin failed because the animals died during the preliminary period of adjustment to the food." Their rats obviously died in a short time, as did mine on the edestin diet, so it is possible that this failure is due to insufficient vitamin B in the diet, especially as they fed 80 % caseinogen successfully. It is not possible to make any definite statement, because there is no way of comparing the respective amounts of vitamin B used, since their yeast (of which 400 mg. were given as a daily ration) may have been relatively stronger or weaker in vitamin B than the marmite used by me.

Exp. 9, in which the rats lived for 16 weeks on a diet containing 20 % of edestin as sole protein, also implies that edestin is a satisfactory constituent, especially as breeding took place at the normal time. It is, however, clear from the experiments described in this paper that for good growth edestin requires more marmite than either caseinogen or egg-albumin. It is possible that some specific amino-acid present to a greater extent in edestin than in other proteins used for feeding experiments is responsible for the amount of marmite required. That there are obvious differences in the metabolism of amino-acids has been shown by Seth and Luck [1925] who found that in the case of histidine there is no increase in the urea content of blood even after 6 hours, while other amino-acids cause appreciable urea production. At the present time one cannot make more than a tentative suggestion on this point, because the analyses of proteins in respect of amino-acids vary so considerably. Since marmite is used in synthetic diets solely as a source of vitamin B, it

can be assumed that more vitamin B is required for the metabolism of edestin, and also, as marmite autoclaved at 120° for 4½–5 hours was equally satisfactory in preventing the kidney symptoms, the antineuritic substance [Chick and Roscoe, 1927] is obviously not the important factor in this case. Presumably the rats obtained sufficient antineuritic substance from excreta to which they had access, or from some constituents of the diet (the butter, or protein which was not pure). It is extraordinary that the older rats did well when only 2 g. marmite were given, and that no kidney abnormalities resulted, especially in view of the work of Osborne and Mendel [1922] demonstrating that the amount of vitamin B required by the rat is proportional to the weight and age of the animal. No doubt when more is known of the complex nature of vitamin B, these discrepancies will disappear, for we are dealing with several factors, not yet completely separated. From the experiments described in this paper the kidney symptoms are undoubtedly characteristic of young rats, possibly analogous to the development of rickets, which disease is only met in young animals.

These experiments support the view that there is a definite relation between the protein and vitamin B of the diet, and further suggest that different proteins may require various amounts of vitamin B in order that they may be properly metabolised. Egg-albumin appears to require less vitamin B than the other proteins used. This may be correlated with the fact that egg-albumin provides for slower growth and therefore a lower proportion of marmite is adequate.

SUMMARY.

1. On a synthetic diet containing 20 % of edestin and 5 g. yeast extract per 100 g. dry solid, many young rats die, showing typical kidney abnormality; older rats are not adversely affected by the diet.
2. These symptoms can be prevented by the addition of more yeast extract to the diet.
3. The "protective" factor in yeast extract is not destroyed by autoclaving at 120° for 4½–5 hours.
4. Edestin requires more yeast extract than either caseinogen or egg-albumin for normal metabolism in young growing rats.

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